

Application No.: 10/602,747

Docket No.: D8200.0004/P004

AMENDMENTS TO THE SPECIFICATION

Page 1, please amend the title as follows:

NATURAL PROMOTERS FOR GENE EXPRESSION AND METABOLIC
MONITORING METHODS FOR THE EXPRESSION OF CODING REGIONS OF
INTEREST IN BACILLUS SPECIES

Page 1, please replace the paragraph beginning at line 5 with the following:

This application is a divisional of U.S. serial no. 09/891,641, filed June 26, 2001 which is now Patent No. 6,617,148 which claims the benefit of U.S. Provisional Application No. 60/214,967, filed June 29, 2000 and of U.S. Provisional Application No. 60/268,320, filed February 13, 2001.

Page 4, please replace the paragraph beginning at line 14 with the following:

Within the context of the present invention the *Bacillus sp.* cell is selected from the species consisting of *Bacillus subtilis subtilis*, *Bacillus thuringiensis*, *Bacillus anthracis*, *Bacillus cereus*, *Bacillus brevis*, *Bacillus megaterium*, *Bacillus intermedius*, *Bacillus thermoamyloliquefaciens*, *Bacillus amyloliquefaciens*, *Bacillus circulans*, *Bacillus licheniformis*, *Bacillus macerans*, *Bacillus sphaericus*, *Bacillus stearothermophilus*, *Bacillus laterosporus*, *Bacillus acidocaldarius*, *Bacillus pumilus*, and *Bacillus pseudofirmus*.

Page 15, please replace the paragraph beginning at line 2 with the following:

The invention identifies a number of genes known in the art as being responsive to various conditions not heretofore appreciated. The identification of these new inducing conditions was made by means of the application of DNA microarray technology to the *Bacillus subtilis subtilis* genome. Any *Bacillus* species may be used, however *Bacillus subtilis subtilis* strain, obtained from *Bacillus* Genetic Stock Center (Ohio State University, Columbus, OH) is preferred.

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Page 19, please replace the paragraph beginning at line 3 with the following:

It will be appreciated by the skilled person that the genes of the present invention have homologues in a variety of *Bacillus* species and the use of the genes for heterologous gene expression and the monitoring of bioreactor health and production are not limited to those genes derived from *Bacillus subtilis subtilis* but extend to homologues in any *Bacillus* species if they are present. For example the invention encompasses homologues derived from species including, but not limited to *Bacillus subtilis subtilis*, *Bacillus thuringiensis*, *Bacillus anthracis*, *Bacillus cereus*, *Bacillus brevis*, *Bacillus megaterium*, *Bacillus intermedius*, *Bacillus thermoamyloliquefaciens*, *Bacillus amyloliquefaciens*, *Bacillus circulans*, *Bacillus licheniformis*, *Bacillus macerans*, *Bacillus sphaericus*, *Bacillus stearothermophilus*, *Bacillus laterosporus*, *Bacillus acidocaldarius*, *Bacillus pumilus*, and *Bacillus pseudofirmus*. Although all of the genes of the present invention have been identified in the *Bacillus subtilis* genome (Kunst et al., *Nature* 390 (6657), 249-256 (1997) homologs of *csn* for example have been identified in *Bacillus circulans*, and *Bacillus ehimensis* (Shimosaka et al., *Appl. Microbiol. Biotechnol.* (2000), 54(3), 354-360; Masson et al., *Gene* (1994), 140(1), 103-7 and in *Bacillus amyloliquefaciens* (Seki et., *Adv. Chitin Sci.* (1997), 2, 284-289.

Page 24, please replace the paragraph beginning at line 2 with the following:

The genes of the present invention may be used to effect the regulated expression of chimeric genes in various *Bacillus* sp. under specific induction conditions or at a specific point in the cell growth cycle. Useful chimeric genes will include the promoter region of any one of the inducible genes defined herein, operably linked to a coding region of interest to be expressed in a *Bacillus* host. Any host that is capable of accommodating the promoter region is suitable including but not limited to *Bacillus subtilis subtilis*, *Bacillus thuringiensis*, *Bacillus anthracis*, *Bacillus cereus*, *Bacillus brevis*, *Bacillus megaterium*, *Bacillus intermedius*, *Bacillus thermoamyloliquefaciens*, *Bacillus amyloliquefaciens*, *Bacillus circulans*, *Bacillus licheniformis*, *Bacillus macerans*, *Bacillus*

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sphaericus, *Bacillus stearothermophilus*, *Bacillus laterosporus*, *Bacillus acidocaldarius*, *Bacillus pumilus*, and *Bacillus pseudofirmus*.

Page 30, please replace the paragraph beginning at line 17 with the following:

Using a *Bacillus subtilis* DNA microarray prepared according to the methods described in Example 1, applicants have identified promoters that can be employed for different level of gene expression in *Bacillus subtilis* and like organisms with oxygen-limiting environment as the induction conditions. This Example describes the identification of anaerobically induced genes and their corresponding promoters in *Bacillus subtilis* when grown in 2xYT 2-X-YF medium. Cells grown at exponential were used.

Page 32, please replace the paragraph beginning at line 16 with the following:

To identify genes induced at stationary phase in the presence of oxygen, the mRNA signals between exponential (log) and one of the stationary samples (T0, T1, or T3) were compared. If the ratio between stationary and log samples was high, it indicated that a particular gene or DNA region was up-regulated at stationary phase. With this DNA microarray technology, many genes were found to have an increased level of mRNA in different stages as shown in Table 3. Genes such as *yegMN*, *csn*, *yvaW*, *yvaX*, *yvaY*, *yncM*, *yryD*, and *yqhIJ* were ~~yqhIJ~~ were all induced in all three stages. Gene such as *yolI*, *yolJ*, *yolK* and *ydjL* were mostly induced at stage T0 and T2. Expression patterns of these genes at stationary phase had not been studied before. The *aco* regions involved in metabolism of acetoin at stationary phase have been previously studied, but only with the DNA microarray technology that they were found to be the highest induced region at T1 stage under this growth conditions. There were quite a few clusters of genes, which were uncharacterized, that showed higher levels of mRNA three hours into the stationary phase. They included *ykfABCD*, *yjmCDEFG*, and *yodLPORST*. In contrast, DNA regions such as *alsT* and *yxeKLMN* showed a reduction in mRNA levels upon entering stationary phase. This data is summarized in Table 3. Table 3 describes a selection of genes or gene clusters that showed an induction or reduction (in parenthesis) in mRNA transcriptional levels at

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stationary phase of *Bacillus subtilis* when grown in Schaeffer's medium supplemented with 0.1% glucose in the presence of oxygen.